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Magnetotactic bacteria selectively synthesize membrane-bound, nanometer-sized, single-domain magnetic particles known as magnetosomes. Because these bacteria have complex nutritional requirements, only one species, Aquaspirillum magnetotacticum, has been grown in pure culture. This bacterium produces approximately twenty intracellular magnetic particles per cell of single-domain size. To synthesize these particles, A. magnetotacticum must possess a highly efficient system(s) to remove iron from the environment. To investigate the mechanism of iron uptake and the synthesis of magnetic particles in this organism, we will construct and screen genomic libraries of A. magnetotacticum for the iron-uptake and magnetosome-synthesizing genes. We will also use the available information on the mechanisms of iron-uptake in other bacteria to identify and characterize analogous systems, related genes, or homologous sequences in this magnetotactic bacterium.

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PROGRESS REPORT ON CONTRACT N00014-89-C-0085

TITLE: Genetic Engineering of Single-Domain Magnetic Particles

PRINCIPAL INVESTIGATOR: Nahid S. Waleh. Molecular Biology Department
SRI International, Menlo Park, CA 94025

START DATE: March 1, 1989

PROGRESS AND PLANNED ACTIVITIES

Screening the Genomic Library for a Siderophore-Mediated Iron-Uptake System

We have constructed and screened a genomic library prepared from the DNA of A. magnetotacticum for the genes of a siderophore-mediated iron-uptake system. A hydroxamate-mediated iron-uptake system had previously been reported to exist in this bacterium. The recombinant cosmids were propagated in an iron-uptake-deficient strain of E. coli host strain. Library clones were plated on a medium containing a dye-iron complex (Schwyn and Neiland, Anal. Biochem. 160: 47, 1987) that turns from blue to orange in the presence of a chelating molecule). In spite of our extensive screening, we were unable to identify a siderophore-producing colony. Out of 10,000 colonies tested, none changed the color of the medium from blue to orange.

One possibility is that the iron-uptake genes are scattered in the chromosome of A. magnetotacticum. In that case one should be able to detect the siderophore or its binding activity in the supernatant culture fluids of the organism. We used the Csaky test for the detection of hydroxamate-type siderophore in the supernatant culture fluids. In this test also, we did not observe any iron-binding activity, even when the supernatant was concentrated by about 20-fold.

Molecular Cloning of a Sequence of A. magnetotaccticum that Allows Growth in the Presence of 2.2'-Dipyridyl

We have also screened the genomic library of A. magnetotacticum for sequences that would allow the growth of the organism in the presence of the chelating agent 2.2'-dipyridyl. We were successful in isolating a 2 Kb DNA fragment that promoted the growth of E. coli entA fepA in the presence of inhibitory concentrations of the chelating agent. This fragment does not mediate any siderophore synthesis as tested by the universal assay medium of Schwyn and Neilands. This fragment, however, complemented the aromatic amino acid requirements and the iron-uptake deficiencies of the E. coli and Salmonella typhimurium strains that lacked a functional aroD gene sequence. In independent experiments, all recombinant cosmids that were selected for their aroD complementation property also carried this DNA sequence as was determined by partial DNA sequencing of the fragments. These results indicate that either the 2 Kb DNA fragment contains one gene and that the aroD gene product of A. magnetotacticum has screened the chelating property, or it codes for two (or more) functional sequences and the aroD activity and the chelating property belong to separate gene products. A restriction map of this fragment has been prepared and its sequence analysis is currently in the progress to resovle these questions.

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Codon Usage in A. magnetotacticum

In order to obtain information about the codons used by A. magnetotacticum, we cloned and sequenced a gene unrelated to iron-uptake genes. The knowledge of codon usage was valuable to us for the construction of more specific probes needed in various screening experiments. The gene we chose for this purpose was the gene analogous to the recA gene of E. coli. The recA gene of E. coli is invoved in homologous recombination and DNA repair. It also regulates the expression of a number of genes scattered in the chromosome. Because of its importance to the cell, we assumed that this gene must have been preserved evolutionarily among bacterial species. We were successful in isolating and characterizing a recA-like sequence from the genomic library of A. magnetotacticum. This gene was highly homologous to the recA gene of E. coli both functionally and structurally.

The entire recA-like gene of A. magnetotacticum was sequenced and analyzed for the frequency of codons used. The codon preference of A. magnetotacticum for certain amino acids was to be markedly different from that of E. coli. For example, A. magnetotacticum has a strong preference for codon TTC for phenylalnine while E. coli appears to have no preference at all.

Cloning the tonB Gene

We have identified a few positive library clones in the hybridization experiments using a high-specific activity, single-stranded tonB sequence as probe. We have sequenced two of these clones up to about 500 bases and have not yet detected any significant homology between these and the tonB sequence. The sequence analyses of other fragments is in progress.

We have constructed primers complementary to the 5' and 3' regions of the tonB gene of E. coli and have used them in PCR reactions to amplify the tonB-like gene of A. magnetotacticum. In these experiments, we obtained three major products, one a fragment with approximately the same size as the PCR product of the tonB gene of E. coli. We have sublconed these fragments into the M13 bacteriophage for sequencing. Our results so far indicate that the cloned fragments are highly unstable in M13. We are currently analyzing more plaques for the identification of stable recombinant molecules.

Screening for Other Iron-Uptake Related Genes

We have conducted Southern blot experiments with the digested DNA of A. magnetotacticum using a number of iron-uptake associated genes of E. coli as probe. The sequences that we have examined so far include, the entire aerobactin operon of E. coli, the receptor gene of ferrichrome-mediated iron-uptake gene (fhuA), a ferrichrome-mediated iron-uptake gene (fhuB), the consensus Fur binding site, the tonB gene, and the btuB gene. In these experiments, we have so far been able to identify fragments that hybridize to the tonB specific probes. The tonB homologous sequences of A. magnetotacticum have been cloned and analyzed as described in the previous section.

PUBLICATIONS

1. A.E. Berson, D.V. Hudson, and N.S. Waleh. 1989. Cloning and Characterization of the recA gene of A. magnetotacticum. Arch. Microbiol. 152:567-571.

2. A.E. Berson, R.M. Peters, and N.S. Waleh. 1990. Nucleotide sequence of the recA gene of A. magnetotacticum. Nucl. Acids Res. (In Press).

PRESENTATIONS

Molecular Cloning of a Sequence of Aquaspirillum magnetotacticum that Allows Growth in the Presence of 2.2'-Dipyridyl. To be presented in the poster session in the American Society for Microbiology in Los Angeles in May of 1990.

TRAINING ACTIVITIES

One undergraduate student.

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Cloning and characterization of the *recA* gene of *Aquaspirillum magnetotacticum**

Amy E. Berson, Debra V. Hudson, and Nahid S. Waleh

Molecular Biology Department, SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025, USA

Abstract. The *recA* gene of *Aquaspirillum magnetotacticum* has been isolated from a genomic library and introduced into a *recA* mutant strain of *Escherichia coli* K12. The cloned gene complemented both the recombination and DNA repair deficiency of the host and its protein product promoted the proteolytic cleavage of the LexA protein. A protein whose molecular weight is similar to that of the RecA protein of *E. coli* was associated with the cloned sequence.

Key words: *RecA* gene – *Aquaspirillum magnetotacticum* – Gene library – Recombinant cosmid

Aquaspirillum magnetotacticum is a Gram-negative freshwater spirillum that synthesizes nanometer-sized, single-domain magnetic particles (for review see Blakemore 1982). We have recently constructed a gene library from the genomic material of this organism, which we have used to complement auxotrophic mutant strains of *Escherichia coli* K12 (Waleh 1988). To investigate whether mutations other than amino acid auxotrophy could be complemented with *A. magnetotacticum* genes, we screened the library for sequences that would complement the *recA* function of *E. coli* K12.

RecA-like sequences have been isolated from a number of bacterial species (West et al. 1983; Pierre and Paoletti 1983; Keener et al. 1984; Ohman et al. 1985; Goldberg and Mekanlaos 1986; Koomey and Falkow 1987). The *recA* gene product in *E. coli* is involved in homologous recombination (Clark 1973) and DNA repair (Hanawalt et al. 1979; Walker 1984). This protein also regulates the expression of a number of unlinked chromosomal genes by promoting the proteolytic cleavage of their repressor molecule, the LexA protein (Walker 1984). The LexA protein is also the repressor of the *recA* gene (Mount 1977).

In this paper, we report the cloning and characterization of the *recA* gene of *A. magnetotacticum*. Hybridization experiments indicate that homology exists between the *recA* sequences of *E. coli* and *A. magnetotacticum*, and complementation studies suggest that the two RecA proteins are functionally similar. A protein whose gel migration pattern is similar to the RecA protein of *E. coli* is produced when recombinant clones are treated with DNA-damaging agents.

* This paper is affectionately dedicated to Prof. John L. Ingraham

Offprint requests to: N. S. Waleh

Materials and methods

Bacteria and plasmids. *Aquaspirillum magnetotacticum* strain MS-1 was provided by BioMagnetech Corporation. Strain HB101, F^- *hsd20* (rB^- mB^-) *recA13 ara-14 proA2 leuB6 thi-1 lacY1 galK2 xyl1 mtl1 supE44 str* λ^-, was used to propagate the gene library. CL142 (K12-Row) was used as the colicin indicator strain (Ozeki et al. 1962). Plasmid pJC859 (provided by John Clark, University of California at Berkeley, Berkeley, California, USA) is a pBR322 derivative and carries the *Escherichia coli recA* gene.

Culture conditions. *A. magnetotacticum* strain MS-1 was grown according to the procedures described by Blakemore et al. (1979). *E. coli* cells were grown in LB liquid or LB agar medium. For the induction of RecA protein, cells were grown in M9 medium supplemented with 0.3% casamino acids and 0.2% thiamine. Ampicillin (amp) was added at the final concentration of 50 µg/ml when required. MMS plates were prepared by spreading 200 µl of a 2% aqueous solution on the surface of LB plates.

Cloning the recA gene. A gene library from DNA of *A. magnetotacticum* was constructed in a broad host-range cosmid c2RB (Bates and Swift 1983) as described previously (Waleh 1988).

Purification of plasmids and DNA fragments. Plasmids were purified by the procedure of Rodriguez and Tait (1983). DNA fragments were purified from low-percentage SEAPLAQUE gels (FMC Corporation, Rockland, ME).

UV survival measurement. Cells were grown in LB-amp liquid medium at 37°C overnight. They were then pelleted by centrifugation and resuspended in an equal volume of TEN (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 150 mM NaCl) buffer. The cell suspension was serially diluted in the above buffer and 0.01 ml volumes of dilutions were spotted on LB agar-amp medium. Plates were placed at a distance of 82 cm from a 15-watt germicidal low-pressure mercury lamp (GE G8T5) and irradiated for the indicated time periods. Plates were wrapped in aluminium foil to prevent

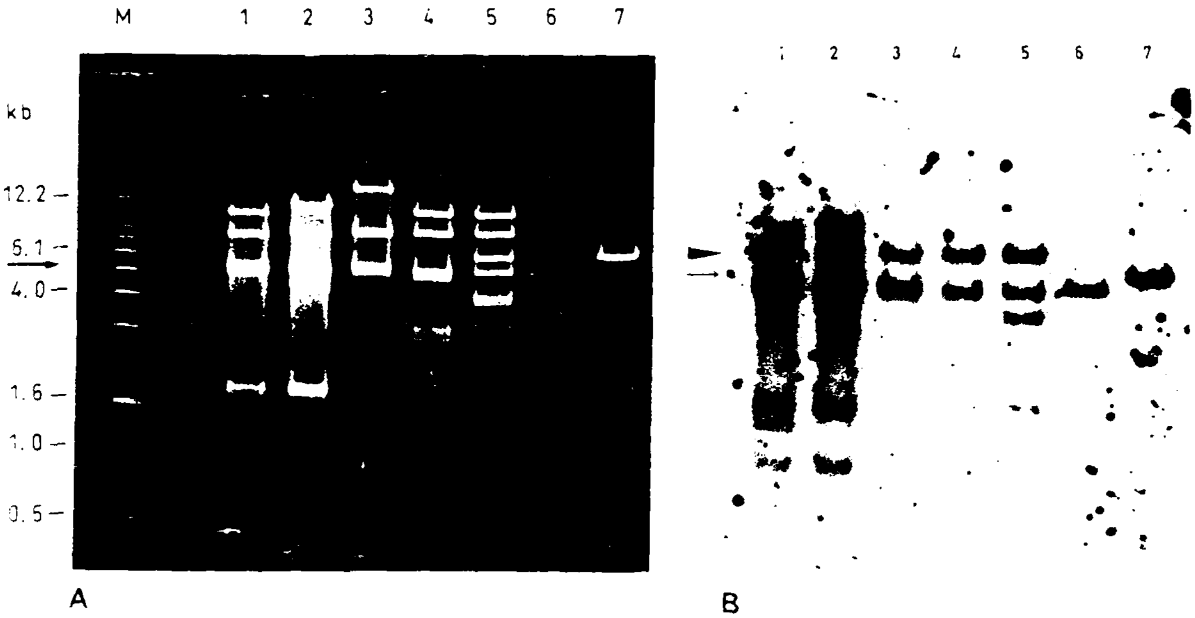

Fig. 1 A, B. *EcoRI* digestion patterns of RecA⁺ recombinant cosmids (*lanes 1–5*), a control RecA recombinant cosmid (*lane 6*), and pJC859 (*lane 7*). The marker fragments, 1 kb ladder obtained from BRL, are shown in lane M. **B** Southern blot hybridization of DNA fragments shown in A using the *EcoRI* digested and ^{32}P-labeled DNA fragments of one of the RecA⁺ recombinant cosmids (shown in *lane 1*) as probe. The vector band is indicated by → and the 8.0 kb *EcoRI* fragment shared by all RecA⁺ recombinant cosmids is shown by ▶

photo-reactivation and incubated at 37 C overnight. The cross-streak method was used for screening a large number of colonies. In this test, overnight-grown cultures were streaked across LB agar-amp plates, and one-half of each streak was irradiated for 30 s. Plates were incubated at 37°C overnight in the dark. The RecA⁻ mutant cells were killed at this UV dose, and the RecA⁺ cells produced a thin film of growth in the irradiated parts of the streak.

Southern blot analysis. Plasmids were digested with *Eco*RI, electrophoresed in a 0.8% agarose gel, denatured in situ, and transferred to nitrocellulose filters as described by Maniatis et al. (1982). *Eco*RI-digested DNA fragments of recombinant cosmids were labeled with [γ-^{32}P]ATP by T4 DNA kinase and were used as probe. The probe from *E. coli recA* gene was prepared by nick-translation using a BRL kit. The nitrocellulose filters were baked in an 80 C vacuum oven for 2 h and hybridized for 22 to 24 h at 45 C in a solution containing 50% formamide, 5×SSC (0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0), 0.8% Denhardt's solution (Maniatis et al. 1982), and 300 μg of heat denatured salmon sperm DNA. Filters were washed at room temperature in 2×SSC – 0.1% sodium dodecyl sulfate (SDS) for 20 min, at 45 C in 0.2×SSC – 0.1% SDS for 30 min, wrapped in Saran wrap, and exposed to X-ray film at −70 C using an intensifying screen (Cronex Hi-Plus).

Colicin test. Colonies were spotted on LB agar plates and were incubated at 37 C. After overnight incubation, cells were killed by exposure to chloroform vapor for 30 min and overlayed with 3 ml soft agar seeded with strain CL142. The colicin-producing colonies produced a zone of inhibition in the lawn of the indicator strain

Induction of RecA protein. Cells were grown in minimal medium in a shaking 37 C incubator to an optical density of about 0.5 – 0.6 at 660 nm. At this time, cells were exposed either to UV light for the indicated periods of time or to mitomycin C added to the cultures at the final concentration of 1 μg/ml. Cells were shaken at 37 C in the dark for two additional hours. Samples were taken at indicated times; cells were pelleted and stored at −20°C.

Polyacrylamide gel electrophoresis. Cell pellets were resuspended in a dye mixture consisting of 1% SDS, 20% glycerol, 40 mM Tris-HCl, pH 6.8, 0.05% bromphenol blue (BPB), and 0.14 M 2-mercaptoethanol, boiled for 5 min in a boiling water bath, and electrophoresed through a 15% acrylamide gel. Cells were stained with Coomassie brilliant blue.

Results

Isolation of the Aquaspirillum magnetotacticum recA gene

The gene library prepared from chromosomal DNA of *A. magnetotacticum* was propagated in HB101, a *recA* mutant strain of *Escherichia coli* K12. This strain is sensitive to DNA-damaging agents, such as MMS, because of its deficiency in homologous recombination and DNA repair functions. Library clones that grow in the presence of MMS should therefore carry sequences that complement the *recA* deficiency and allow the growth of their host strain. Of 542 amp-resistant (amp^r) clones tested, we found 5 that grew in the presence of MMS. Plasmid analysis indicated that all clones carried recombinant cosmids with inserts that were between 26 – 35 kb in size. The *EcoRI* digestion patterns of the recombinant cosmids is shown in Fig. 1A. *EcoRI* digestion separates the insert DNA from the vector DNA. Southern hybridization analysis of the *EcoRI* digests of the recombinant cosmids using on the RecA⁺ clones as probe

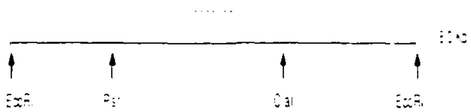

Fig. 2. Preliminary restriciton map of the 8.0 kb *EcoRI* fragment carrying the *recA* gene of *Aquaspirillum magnetotacticum*. The dashed line shows the approximate location of the *recA* gene as suggested by subcloning experiments described in the text

(Fig. 1B) demonstrates that all five RecA$^+$ recombinant cosmids, in addition to the vector band, share a fragment of about 8 kb (lanes 1 – 5). As expected, no DNA homology (except for the cosmid DNA) was detected between the fragment inserts of the RecA$^+$ clones and those of an amp^r but MMS-sensitive (MMS^s) clone of the library (lane 6) that was used as negative control. *EcoRI* cleaves pJC859 into two fragments of 5750 and 1850 bp in size (Fig. 1A, lane 7). In the hybridization experiment, however, only the larger fragment shows homology with the *recA* sequence of *A. magnetotacticum* (Fig. 1B, lane 7). This fragment carries about 80% of the *E. coli recA* gene, including the promotor and the operator sequence (Sancar et al. 1980). When *E. coli* sequence was used as probe, only the 8-kb fragment of the RecA$^+$ recombinant cosmids, hybridized with the labeled probe (data not shown).

Restriction mapping of the A. magnetotacticum recA gene

The restriction digestion and Southern hybridization analysis described above indicated that all five RecA$^+$ recombinant cosmids shared a fragment of about 8 kb that hybridized with the labeled *E. coli recA* sequence. This fragment was purified and cloned into the *EcoRI* site of pBR322. The recombinant plasmid thus formed was used to transform HB101. All amp^r transformants were found to be MMS^r. When 20 of the amp^r/MMS^r clones were tested for their sensitivity to UV light, all were found to be also UV resistant (UV^r). These results indicated that the 8-kb fragment carries the *recA* sequence of *A. magnetotacticum*.

The 8-kb fragment was digested with *PstI* and/or *ClaI* endonucleases and the resulting fragments were cloned into pBR322 digested with the same endonucleases. Upon screening of the transformants and identification of the fragments that conferred MMS^r and UV^r, the *recA* sequence of *A. magnetotacticum* was localized to a fragment of about 3-kb between *PstI* and *ClaI* restriction sites (Fig. 2). The 3-kb fragment was further purified and ligated with pBR322 digested with *PstI* and *ClaI* endonucleases. The ligated DNA molecules were used to transform HB101. Since *PstI* and *ClaI* digestions inactivate both antibiotic resistance markers of pBR322, transformants were selected for MMS^r and were further tested for UV^r. All MMS^r transformants were found to be UV^r and to carry the plasmid for the expected size. This plasmid construct was designated pNW300.

Complementation studies with A. magnetotacticum recA gene

The recombination proficiency of HB101 clones carrying the *recA* gene of *A. magnetotacticum* was determined by measuring the plating efficiency of a *red$^-$ gam$^-$* mutant strain of bacteriophage λ (λ Fec$^-$ phenotype). This mutant phage requires the recombination activity of the RecA protein for its growth in *E. coli* cells (Manly et al. 1969). All

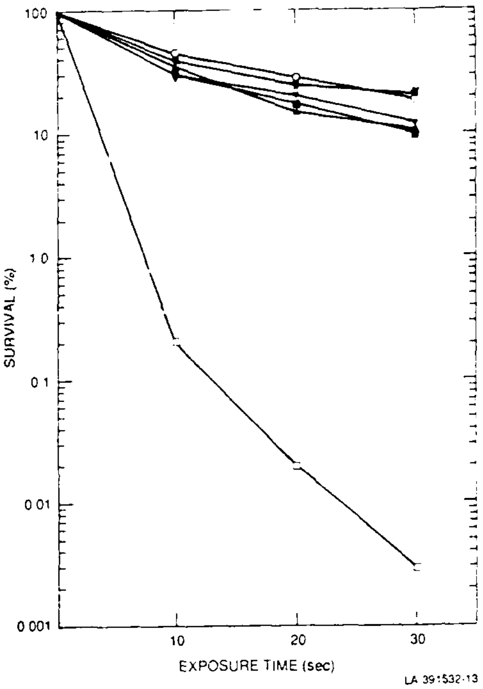

Fig. 3. UV survival of cells carrying the *recA* gene of *A. magnetotacticum*. Symbols: ×, ▲, ■, ▼, and ●, RecA$^+$ library clones; ○, HB101 (pJC859); and □, a RecA$^-$ library clone

clones carrying the *recA* gene of *A. magnetotacticum* supported the growth of λ *red$^-$ gam$^-$* phage. The plating efficiency of the phage on these RecA$^+$ library clones was the same – about 30% of that obtained with strain HB101 (pJC859), which carries the *E. coli recA* gene. Strain HB101 alone did not support the growth of λ *red$^-$ gam$^-$*, and only few plaques were formed on plates with the lower dilutions of phage.

Because of their deficiency in DNA repair, the *recA* mutant strains of *E. coli* are sensitive to UV light. To see whether the *recA* gene of *A. magnetotacticum* can complement the mutant function, we examined the ability of the RecA$^+$ recombinant cosmids to repair the UV-damaged DNA of their *recA$^-$* host. In these experiments, *E. coli* strain HB101, which carried pJC859, and a library clone picked at random were used as positive and negative controls, respectively. Quantitative UV survival measurements (Fig. 3) indicated that all recombinant cosmids with the cloned sequence conferred UV^r upon their host. The extent of protection in each case was similar to that confered by pJC859. No protection was detected by a control recombinant cosmid that was amp^r and MMS^s.

The RecA protein of *E. coli* promotes the proteolytic cleavage of the LexA protein which negatively regulates the expression of a number of unlinked chromosomal genes of *E. coli* (Walker 1984). Since LexA is also the colicin E1 gene repressor, we examined whether the RecA protein of *A. magnetotacticum* promotes the cleavage of the LexA protein and induces the expression of the colicin E1 gene. For this purpose, strains HB101, HB101 (pJC859) and HB101 (pNW300) were transformed with plasmid pNP12 (Waleh and Johnson 1985). This pBR322-derived plasmid confers resistance to tetracycline and carries the entire colicin E1 operon. Transformants were selected for tetracycline resis-

Table 1. Colicin production after induction of pNW300 and pJC859 with mitomycin C (MC)

Strains carrying plasmid pNP12	Colicin titer	
	−MC	+MC
HB101 (pNW300)	0.01	1
HB101 (pJC859)	0.01	10
HB101	0	0

Results represent colicin titer $\times 10^{-2}$. The colicin titer is defined as the reciprocal of the last dilution giving noticeable clearing of the indicator lawn

tance and were tested for colicin production. Of 8 colonies tested, all pNP12-carrying clones of HB101 (pNW300) produced colicin. The zones of inhibition produced by these clones, however, were smaller than those produced by HB101 clones carrying plasmids pNP12 and pJC859 (6 mm versus 11 mm). As expected, none of the HB101 (pNP12) colonies produced any colicin. One transformant colony from each transformation set was picked and tested for the production of colicin in the presence of mitomycin C. Cultures, grown to midlog phase, were divided in half. To one half, mitomycin C was added at the final concentration of 1 μg ml; the other half was used as control. After 2 h of incubation, samples were taken, cells were pelleted, and the supernatants were titrated for colicin activity on a colicin-sensitive strain, CL142. The results (presented in Table 1) indicated that the amount of colicin produced by pNP12-carrying strain of HB101 (pNW300) was increased 100× upon treatment with mitomycin C. This amount of colicin was, however, tenfold less than the one produced by the pNP12-carrying strain of HB101 (pJC859) that carries the native gene. No colicin activity was detected in the HB101 (pNP12) culture supernatant.

Protein analysis of strain HB101 (pNW300)

Soluble protein extracts were prepared from untreated and mitomycin C-treated or UV-irradiated cells of HB101 (pNW300), HB101 (pJC859), and HB101; they were electrophoresed in a polyacrylamide gel and stained with Coomassie brilliant blue (Fig. 4). Treatment of HB101 (pNW300) with either mitomycin C or UV induced the production of a protein that migrated near the position of *E. coli* RecA protein. This protein was absent in extracts of strain HB101.

Discussion

We have cloned and partially characterized a DNA fragment of the genome of *Aquaspirillum magnetotacticum* that codes for a protein analogous to the *recA* gene product of *Escherichia coli* K12. The screening technique we used was based on heterologous complementation of an *E. coli recA* mutant that was first described by Better and Helinski (1983) for cloning the *recA* gene of *Rhizobium meliloti*. This technique was later used by others (Keener et al. 1984; Ohman et al. 1985; Goldberg and Mekalanos 1986; Koomy and Falkow 1987) to clone analogous *recA* sequences from other bacterial species.

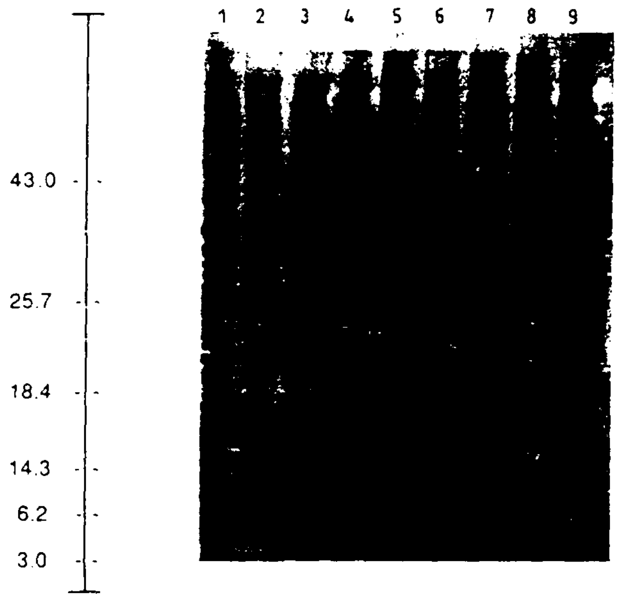

Fig. 4. Protein analysis of plasmid pNW300. Cells were grown in minimal medium to midlog phase when they were treated with mitomycin C at 1 μg ml, or UV irradiated for 30 s. After 2 h of incubation at 37 C, cells were pelleted and treated as described under Materials and methods. *Lanes 1–3, 4–6,* and *7–9* show protein samples from control, mitomycin C-treated, and UV-irradiated cells of HB101, HB101 (pNW300), and HB101 (pJC859), respectively. Molecular weight protein standards are given $\times 10^{-3}$

DNA hybridization experiments demonstrate that significant homology exists between the *recA* sequence of *A. magnetotacticum* and that of *E. coli* K12. This homology appears to be mainly at the amino-terminal portion of the two sequences, however, as evidenced by hybridization of probes complementary to the *recA* of *A. magnetotacticum* with the fragment that carried the amino-terminal and nearly 80% of the total *recA* sequence of *E. coli* (Fig. 1).

The RecA protein of *A. magnetotacticum* restored recombination proficiency in the *E. coli recA* mutant host. This was demonstrated by increased plating efficiency of the λ Fec⁻, which requires the *recA* function of the host for growth. The lower number of λ plaques observed with the RecA protein of *A. magnetotacticum* may be due to inefficient expression from the heterologous promoter or, alternatively, due to instability of the RecA protein in a foreign host. Indeed, in crude cell extracts, the RecA protein of *A. magnetotacticum* appears to be unstable and degrades rapidly upon short-term storage.

The RecA protein of *A. magnetotacticum* increased cell viability of the host to wild-type levels in response to UV exposure. HB101 cells with *recA* gene of *A. magnetotacticum* were as UV resistant as those carrying the native sequence. Similar levels of protection have been reported in heterologous complementation studies with the RecA proteins of *Proteus vulgaris*, *Shigella flexneri*, *Erwinia carotovora*, and *E. coli* B/r (West et al. 1983; Keener et al. 1984). However, RecA protein of *R. meliloti* has only partially suppressed the UV sensitivity of an *E. coli recA* mutant (Better and Helinski 1983).

The most interesting results were the findings that the *recA* of *A. magnetotacticum* not only recognizes the LexA protein *E. coli* but that the *recA* itself may be regulated by

this repressor molecule. Our colicin induction experiments clearly demonstrate that the RecA of *A. magnetotacticum* promotes the cleavage of the LexA repressor, which leads to the derepression of the colicin E1 operon. Moreover, the finding that both UV and mitomycin C increased the level of the RecA protein of *A. magnetotacticum* – a response observed with *E. coli* RecA – strongly suggest that the cloned *recA* gene is regulated by the LexA protein of *E. coli*. Whether a LexA-like protein exists in the native host and whether it regulates the expression of the *recA* gene of *A. magnetotacticum* remains to be demonstrated.

Our results and those of the other investigators discussed above provide compelling evidence that the RecA protein is structurally and functionally preserved among Gram-negative bacteria. DNA and amino acid sequence analysis of RecA from various species should provide valuable information about the history of the evolution of this important multifunctional bacterial protein. DNA sequence analysis of the *recA* gene of *A. magnetotacticum* is currently in progress.

Acknowledgements. This work was supported by contract (N00014-89-C-0085) from the Office of Naval Research and SRI International R&D project 391D32 SEE. *A. magnetotacticum* was provided by BioMagnetech Corporation.

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Nucleotide sequence of *recA* gene of *Aquaspirillum magnetotacticum*

Amy E. Berson, [illegible]rice R. Peters and Nahid S. Waleh

Department of Molecular Biology, SRI International
Menlo Park, CA 94025, USA

We have determined the nucleotide sequence of the *recA* gene of *A. magnetotacticum*. The coding region has 1032 base pairs, specifying 344 amino acids. The deduced protein has a Mr of 36,750, which is consistant with our previously reported estimated value (1). In the 5' non-coding region, there is a potential ribosome binding site (underlined) and an incomplete SOS box (boxed).

The nucleotide sequence shows 61.6% homology with the *recA* sequence of *E. coli* (2). The amino acid residues essential for the recombinase, protease and ATPase functions of the *E. coli* recA protein (3-5) are either conserved in *A. magnetotacticum* or are substituted with similar amino acids. Of 24 amino acid residues believed to be the ATP binding domain of the *E. coli* RecA protein (6), 15 are conserved in the *A. Magnetotacticum* protein. For identical amino acids, the codons used by *A. magnetotacticum* were often found to be different from those used by *E. coli*.

```
TTCTCCTCTCGTTCGTGTACGGTATTCCTCATTGCGGAACCGCCGGTTCGGGATAGATGAAGGGGAACGG  -42
CCATGTCTCAGGCTGCATTGCGTCTCGTGGACAAGGATACCATGGATAGACAGAAGGCTTTGGAAGCTGC   29
                                          M  D  R  Q  K  A  L  E  A  A
CGTCAGCCAGATCGAGCGGGCATTCGGCAAGGGCTCCATCATGAAGCTGGGCGGCAAGGATCAGGTGGTC   99
 V  S  Q  I  E  R  A  F  G  K  G  S  I  M  K  L  G  G  K  D  Q  V  V
GAGACCGAAGTGGTCTCCACCCGGATCCTGGGCCTTGATGTGGCGCTCGGCATCGGCGGCGTTCCGCGCG  128
E  T  E  V  V  S  T  R  I  L  G  L  D  V  A  L  G  I  G  G  V  P  R  G
GCCGTATCATCGAGGTCTATGGCCCGGAAAGCTCGGGCAAGACCACCCTGGCGCTGCACATCATCGCCGA  198
  R  I  I  E  V  Y  G  P  E  S  S  G  K  T  T  L  A  L  H  I  I  A  E
GGCGCAGAAGAAGGGCGGCACCTGCGCCTTCGTCGATGCCGAACACGCGCTTGACCCCTCCTATGCCCGT  268
 A  Q  K  K  G  G  T  C  A  F  V  D  A  E  H  A  L  D  P  S  Y  A  R
AAGCTGGGCGCGCTGGACGAGCTGCTGATCAGCGAGCCCGACGCTGGCGAGCAGGCCCTGGAAATCGCCG  338
K  L  G  A  L  D  E  L  L  I  S  E  P  D  A  G  E  Q  A  L  E  I  A  D
ACACCCTGGTACGCCCCGGCGCCGTGGACGTTCTGGTGGTGGATTCGGTGGCCGCATTGGTGCCCCGCGG  408
  T  L  V  R  P  G  A  V  D  V  L  V  V  D  S  V  A  A  L  V  P  R  G
CGAGCTGGAAGGCGAGATGGGCGACAACCATATGGGCCTGCACGCCCGCCTGATGAGCCAGGCGCTGCGC  478
 E  L  E  G  E  M  G  D  N  H  M  G  L  H  A  R  L  M  S  Q  A  L  R
AAGCTGACCGGTTCGGTATCCAAGTCCAAAACCATCGTCATCTTCATCAACCAGATCCGCATGAAGATCG  548
K  L  T  G  S  V  S  K  S  K  T  I  V  I  F  I  N  Q  I  R  M  K  I  G
GCGTGATGTTCGGCAATCCCGAGACCACCACCGGCGGCAACGCGCTCAAGTTCTACGCCTCGGTGCGCAT  618
  V  M  F  G  N  P  E  T  T  T  G  G  N  A  L  K  F  Y  A  S  V  R  M
GGAGATCCGCCGCGTCGGCGCCATCAAGGACAGGGACGAGGTCGTGGGCAACCAGACCCGCGTCAAGGTG  688
 E  I  R  R  V  G  A  I  K  D  R  D  E  V  V  G  N  Q  T  R  V  K  V
GTGAAGAACAAGCTGGCTCCGCCGTTCAAGGTGGTGGACTTCGACATCATGTATGGCGAAGGCATCTCCA  758
V  K  N  K  L  A  P  P  F  K  V  V  D  F  D  I  M  Y  G  E  G  I  S  K
AGATGGGTGAGCTCATCGATCTGGGCGTCAAGGCCAATGTGGTGAAGAAATCGGGGGCCTGGTTCTCCTA  828
  M  G  E  L  I  D  L  G  V  K  A  N  V  V  K  K  S  G  A  W  F  S  Y
CAACTCCACCCGCATCGGCCAGGGCCGCGAGAACGCCAAGCAGTTCCTGCGCGACAATCCGGCCATGGCC  898
 N  S  T  R  I  G  Q  G  R  E  N  A  K  Q  F  L  R  D  N  P  A  M  A
GCCGAGATCGAAGGCGCCATCCGCCAGAATGCCGGCCTCATCTCCGAGGCCCTGGCCGCGGTCCCGGACC  968
A  E  I  E  G  A  I  R  Q  N  A  G  L  I  S  E  A  L  A  A  V  P  D  L
TGGACGGCACGCCGGTCGCGGAATAACCCTCGCGCGGGTGCGAAAACCACAGGGCCACCCGCGGCAACAC 1038
  D  G  T  P  V  A  E  .
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